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(54) Title: METHODS FOR THE TREATMENT OF ACID ASPIRATION-INDUCED ACUTE LUNG INJURY

(57) Abstract

Lung injury from acid aspiration is treated by administering an IL-8-binding substance, such as anti-IL-8-antibody, to the patient following the acid aspiration.

METHODS FOR THE TREATMENT OF ACID
ASPIRATION-INDUCED ACUTE LUNG INJURY

The present invention is a continuation-in-part of Application Serial No. 08/377,077, filed on January 23, 1995, the full disclosure of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a method for treating and protecting patient lungs exposed to acid resulting from the aspiration of gastric contents or other causes.

The aspiration of gastric acid can occur, for example, when an unconscious patient regurgitates the stomach contents into the lungs. Acid aspiration is the second leading cause of the adult respiratory distress syndrome (ARDS), a syndrome which is characterized by injury to the pulmonary endothelium and the alveolar epithelium with leakage of blood and plasma into the interstitial and intra-alveolar spaces. The mortality from acid-induced ARDS range from 40% to 50%. No adequate treatments are presently available.

For these reasons, it would be desirable to provide methods for the treatment of patients exposed to acid aspiration into the lungs and at risk of developing ARDS. It would be further desirable if the methods could reduce damage to the pulmonary endothelium and alveolar epithelium, particularly when the treatment is given within one hour following the acid injury.

2. Description of the Background Art

Mulligan et al. (1993) J. IMMUNOL. 150:5585 describes the use of anti-IL-8 antibodies to treat immunologically induced lung inflammation. Sekido et al. (1993) NATURE 365:654 shows that anti-IL-8 antibodies are able to reduce reperfusion injuries in ischemic lung tissue in rabbits. Anti-IL-8 antibodies have been shown to inhibit neutrophil influx in an endotoxin-induced pleurisy model in rabbits. Broaddus, et al. (1994) J. IMMUNOL. 152:2960-2967. Elevated levels of IL-8 are present and cause neutrophil influx in patients suffering from adult respiratory distress syndrome. Miller et al. (1992) AM. REV. RESPIR. DIS. 146:427-432. The neutralization of TNF-α (which leads to IL-8 production in many cells) has been shown to reduce lung injury caused by acid aspiration. Goldman et al. (1990) ANN. SURG. 212:513-520. A possible mechanism of acid aspiration-induced lung injury relies on the release of a variety of chemotactic and inflammatory molecules. The chemotactic and inflammatory molecules, in turn, recruit neutrophils which induce injury upon binding to or migrating through the pulmonary capillary endothelium. Such a mechanism is discussed in a number of publications, including Ishii et al. (1989) PROSTAGLANDINS LEUKOT. ESSENT. FATTY ACIDS 37:65-70; Goldman et al. (1990), *supra.*; Goldman et al. (1991) J. APPL. PHYSIOL. 70:1511-1517; Goldman et al. (1992) SURGERY 111:55-61; Fowler et al. (1987) AM. REV. RESPIR. DIS. 136: 1225-1231; Kindt et al. (1991) J. APPL. PHYSIOL. 70:1575-1585; and Miller et al. (1992), *supra.* IL-8 has been proposed as a major chemotactic factor for recruitment of neutrophils to extravascular sites of inflammation, including those in the lungs. Colditz et al. (1990) J. LEUKOC. BIOL. 48:129-137 and Kunkel et al. (1991) EXP. LUNG RES. 17:17-23.

SUMMARY OF THE INVENTION

According to the present invention, the magnitude of acute lung injury following acid aspiration is reduced or eliminated by the systemic administration
5   of an interleukin-8 (IL-8)-binding substance to the patient. The IL-8-binding substance is selected to neutralize free-IL-8 released from cells exposed to the acid. The neutralization of IL-8 inhibits or prevents influx of neutrophils from circulation into the
10   extravascular space of the lung, and it is believed that reduction of neutrophil recruitment by the IL-8-binding substance lessens the cellular damage. The exemplary IL-8-binding substance is anti-IL-8 antibody. The IL-8-binding substance is administered to the patient as
15   rapidly as possible following acid aspiration, but such administration can be delayed for as long as one hour without a substantial loss of therapeutical effectiveness.

20   ## BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A and 1B. Alveolar-arterial oxygen tension difference in the positive control, pretreatment, treatment, and negative control groups over 6 h (Fig. 1A) and over 24 h (Fig. 1B). In the 6 h experiments, the
25   alveolar-arterial oxygen tension difference in the pretreatment and treatment groups was significantly less than that in the positive control group from 2 h onwards and was no different from that in the negative control group. In the 24 h experiments, the alveolar-arterial
30   oxygen tension difference was significantly less in the treatment (long-term) group than in the positive control (long-term) group by 2 h and remained low for 24 h. All the rabbits (n = 3) in the positive control (long-term) group died at 12-14 h. Data are means $\pm$ SEM, * $p < 0.05$
35   versus the negative control group (Fig. 1A) or the treatment (long-term) group (Fig. 1B), $\dagger$ $p < 0.05$ versus

the pretreatment group (Fig. 1A), ‡p<0.05 versus the treatment group (Fig. 1A).

Figs. 2A and 2B. The extravascular lung water (a quantitative index of pulmonary edema) in the positive control, pretreatment, treatment, and negative control groups at 6 h (Fig. 2A) and in the positive control (long-term) group at 12-14 h and treatment (long-term) group at 24 h (Fig. 2B). In the short-term studies, the extravascular lung water in the pretreatment and treatment groups was 35% less than in the positive control groups and no different from that in the negative control group. The extravascular lung water of a normal uninstilled rabbit lung is 3.2 g water/g dry lung. In the long-term studies, the extravascular lung water was 100% lower in the treatment (long-term) group than in the positive control (long-term) group. Data are means ± SD, *p<0.05 versus the positive control group (Fig 2A) or the positive control (long-term) group (Fig. 2B).

Figs. 3A and 3B. Endothelial permeability in the lung measured as the accumulation of the vascular protein tracer, ^{131}I-albumin, in the extravascular spaces of the lung and expressed as extravascular plasma equivalents in the positive control, pretreatment, treatment, and negative control groups at 6 h (Fig. 3A), and in the positive control (long-term) group at 12-14 h and the treatment (long-term) group at 24 h (Fig. 3B). In the short-term studied, the extravascular plasma equivalents were decreased by 70% in the pretreatment and treatment groups compared to the positive control group at 6 h. The same reduction in extravascular plasma equivalents was observed in the treatment (long-term) group at 24 h compared to the positive control (long-term) group at 12-14 h. Data are means ± SD, *p<0.05 versus the positive control group (Fig. 3A) or the positive control (long-term) group (Fig. 3B), #p<0.05 versus the negative control group (Fig. 3A).

Figs. 4A and 4B. The number of neutrophils lavaged from the air spaces of rabbits in the positive control, pretreatment, treatment, and negative control groups at 6 h (Fig. 4A) and in the positive control
5 (long-term) group at 12-14 h and the treatment (long-term) group at 24 h (Fig. 4B). In the short-term studies, the number of neutrophils was 50% lower in the pretreatment and treatment groups than in the positive control group and no different from that in the negative
10 control group at 6 h. In the long-term studies, the number of neutrophils was more than 75% lower in the treatment (long-term) group at 24 h than in the positive control (long-term) group at 12-14 h. Data are means ± SD, $p < 0.05$ versus the positive control group (Fig. 4A) or
15 the positive control (long-term) group (Fig. 4B).

DETAILED DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention is based at least in part on the discovery that acid-induced lung injury is
20 mediated by neutrophils recruited to the lung by interleukin-8 (IL-8)-dependent mechanisms. It is believed that direct injury of the lung from the aspirated gastric acid is limited in extent, perhaps because the acid is rapidly neutralized after entering
25 the lungs. Acid aspiration, however, stimulates the release of substantial quantities of IL-8 into the airspaces from a variety of cells in the lung, including airway epithelial cells, alveolar epithelial cells, and alveolar macrophages. Once generated in the airspaces,
30 IL-8 is believed to diffuse toward the pulmonary endothelium, thereby establishing a chemotactic gradient for neutrophils and possibly binding to the luminal endothelial surface where the IL-8 could interact with circulating neutrophils. The interaction of IL-8 with
35 neutrophils then induces up regulation of neutrophil adhesion molecules on the endothelium, neutrophil migration through the activated endothelium, and priming

of neutrophils for activations by other mechanisms. Neutrophils recruited to the lung in this manner are believed to be responsible for most of the endothelial injury that results in formation of a protein-rich

5 pulmonary edema fluid that is characteristic of acid-aspiration acute lung injury.

The present invention relies on the binding of free IL-8 released from acid-stimulated airway epithelial cells, alveolar epithelial cells, and alveolar

10 macrophages. Binding preferably occurs through a region of the IL-8 molecule which binds to the IL-8-receptor on the neutrophils, thus directly blocking binding and recruitment of the neutrophils by the released IL-8. Binding should occur with an affinity of at least about

15 10^7 M^{-1}, preferably at least 10^8 M^{-1}.

Suitable binding substances for inhibiting binding between IL-8 and circulating neutrophils include anti-IL-8 antibodies, and fragments thereof; an IL-8-receptor protein, or fragment or analogue thereof; or any

20 other protein, glycoprotein, carbohydrate, small molecule, or the like, which is able to disrupt binding between the released IL-8 and the circulating neutrophils. The IL-8-binding substance will preferably bind to the IL-8 molecule at or adjacent to the region on

25 the molecule which binds to the IL-8-receptor on the neutrophils. By binding directly to this region, all interaction between the IL-8 molecule and the neutrophil will be blocked. By binding close to this region, sufficient stearic hinderance may be provided in order to

30 effectively inhibit neutrophil recruitment by the released IL-8.

The presently preferred IL-8-binding substance is neutralizing antibody to the IL-8 molecule. By "neutralizing," it is meant that the antibody will be

35 able to neutralize, i.e. inhibit, binding between the IL-8 molecule and the circulating neutrophils. Such antibodies may be prepared by employing IL-8, or an IL-8-

fragment, as an immunogen in conventional techniques for preparing polyclonal or monoclonal antibodies. Such techniques are well described in the scientific and patent literature. See, for example, *Antibodies: A*

5   LABORATORY MANUAL, Harlow and Lane Eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). A particular technique for preparing neutralizing monoclonal antibodies is described in Broaddus, et al. (1994) J. IMMUNOL. 152:2960-2967.

10   The IL-8-binding substance will be systemically, usually intravenously, administered to the patient as quickly as possible after occurrence of the acid aspiration. Depending on the nature of the particular substance being administered, the total dosage

15 may vary from 1 μg/kg of body weight to 10 mg/kg of body weight. In the exemplary case of monoclonal antibodies, the dosage will typically vary from 1 mg/kg to 10 mg/kg, with the total dosage being administered continuously, as a single bolus or as multiple boluses over time. While

20 intravenous administration is preferred, the IL-8 binding substance could be administered by other systemic routes, such as intratracheally.

          Depending on the intended route of delivery, the IL-8-binding substance will be incorporated into a

25 suitable pharmaceutical composition including the desired dosage of the binding substance. Such compositions will usually include a pharmaceutically acceptable carrier, which can be any compatible, non-toxic substance suitable to deliver the IL-8-binding substance to the patient.

30 Sterile water, alcohol, fats, waxes, and inert solids, may be used as the carrier. Pharmaceutically acceptable adjuvants, buffering agents, and the like, may also be incorporated into the pharmaceutical compositions. Such compositions will be suitable for parenteral

35 administration, i.e. intravenous or for pulmonary (intratracheal) delivery. The preparation of such pharmaceutical compositions is well known in the art and

8

described in references, such as Remington's PHARMACEUTICAL
SCIENCES, Gennaro, Ed., Mack Publishing Co., Easton,
Pennsylvania 18042, 18th Ed., 1990.

   The following examples are offered by way of
5 illustration, not by illustration, not by way of
limitation.

EXPERIMENTAL

METHODS

10 **Animals, Surgical Preparations and Ventilation**

   Male New Zealand White rabbits (n = 32,
weighing 2.5-3.5 kg, Nitabell, Hayward, CA., USA) were
surgically prepared as described earlier. Folkesson et
al. (1994) AM. J. RESPIR. CRIT. CARE MED. 150:1482-1486.
15 Briefly, the rabbits were initially anesthetized using 4%
halothane in 100% O_2; the anesthesia was then maintained
with 0.8% halothane in 100% O_2. Pancuronium bromide (0.3
mg/h x kg body weight; Pavulon®, Organon Inc., West
Orange, N.J., USA) was given intravenously for
20 neuromuscular blockade.

   A 22-gauge Angiocath® was inserted in the
marginal ear vein for administering fluid and drugs. A
PE-90 catheter was inserted in the right carotid artery
to monitor systemic blood pressure and to obtain blood
25 samples. A 4.0 mm ID endotracheal tube was inserted
through a tracheostomy. The rabbits were maintained in
the prone position during the experiments and ventilated
with a constant-volume piston pump (Harvard Apparatus
Co., Dover, MA., USA) with an inspired oxygen fraction of
30 1.0 and with a peak airway pressure of 15-18 cm H_2O during
the baseline period, and supplemented with a positive
end-expiratory pressure of 4 cm H_2O. During the baseline
period, the respiratory rate was adjusted to maintain the
arterial PCO_2 between 35-40 mm Hg. Thereafter, the
35 ventilator settings were kept constant throughout the
experiment.

Preparation of the Instillate

A solution of 100 mOsm/kg of NaCl (1/3 normal saline) was prepared with isotonic 0.9% saline and distilled water. The 1/3 normal osmolality was chosen to
5 match the osmolality of gastric aspirates. Then, hydrochloric acid (HCl) was added to the solution and titrated to a pH of 1.5. In the negative control studies, 1/3 normal saline was used as the instillate. Evans blue dye (1 mg, Aldrich Chemical Company Inc.,
10 Milwaukee, WI., USA) was added to all instillates in order to confirm at post-mortem examination that the instilled fluid was distributed equally to both lungs.

Generation of the Monoclonal Antibody to Rabbit rIL-8

15   The generation of the monoclonal antibody to rabbit rIL-8 (ARIL8.2) has been described in detail. Broaddus et al. (1994) J. IMMUNOL. 152:2960-2967. ARIL8.2 was used because of its ability to recognize rabbit IL-8, to inhibit binding of ^{125}I-labeled rabbit rIL-8 to the IL-8
20 receptor, to block rabbit rIL-8-induced signal transduction via the IL-8 receptor, and to inhibit rabbit rIL-8-induced chemotactic activity for rabbit neutrophils. ARIL8.2 had a high affinity for rabbit IL-8 ($K_d = 0.42$ nM). ARIL8.2 did cross-react with human IL-8,
25 but not with closely related cytokines (hMGSA, platelet factor-4, β-thromboglobulin), other human cytokines (IL-1β, TNF-α), or other chemotactic factors (FMLP, C5a). The antibody preparation was sterile filtered and endotoxin was undetectable by Limulus assay.

30

General Experimental Protocol

In all experiments, after the surgical preparations, a 1 h baseline of stable heart rate, systemic blood pressure, and arterial blood gases was
35 required before the instillation. Fifteen minutes into the baseline period, 3 μCi ^{131}I-labeled human serum albumin (^{131}I-albumin, Frosst Laboratories) was injected

intravenously as a vascular tracer protein. Blood
samples were obtained every 15 minutes for the remaining
45 minutes of the baseline period. The vascular tracer
was used to calculate the flux of plasma protein into the
5 extravascular spaces of the lung.

For the instillation, a tubing (5 Fr.,
Accumark® Premarked Feeding Catheter, Concord/Portex,
Keene, NH., USA) was gently passed through the tracheal
tube until is was placed approximately 1 cm above the
10 carina. Then, HCl or 1/3 normal saline (4 ml/kg body
weight) was instilled into both lungs over 3 minutes.
After the instillation was completed, the tubing was
withdrawn.

Thirty minutes after the instillation and
15 hourly thereafter during the 6 h or 24 h experimental
period, blood was sampled.

At the end of the 6 h or 24 h experiments, the
abdomen was opened and the rabbit was exsanguinated by
transection of the abdominal aorta. The lungs were
20 removed through a median sternotomy. An alveolar sample
was aspirated via sampling catheter gently passed through
the trachea to a wedged position in a distal airway.
Then, the left lung was clamped at the main bronchus for
later use in extravascular lung water and tracer protein
25 measurements (see below). The right lung was then
lavaged 2 times, using 6 ml of isoosmolar 0.9% NaCl
containing 12 mM lidocaine (Sigma Biochemicals, St.
Louis, MO., USA) each time.

The radioactivity of the samples was measured.
30 Total and differential cell counts were measured on the
blood and bronchoalveolar lavage samples. The cells were
counted as cells per ml lavage and then multiplied by the
lavage column used (12 ml). Free, unbound IL-8
concentrations were measured in the plasma samples and
35 the alveolar samples.

By trichloracetic acid (TCA) precipitation of
the instillates and selected samples from each

experiment, it was established that the vascular tracer
^{131}I remained more than 98% bound to protein.

Specific Experimental Protocol

There were six experimental groups. Three of
these groups received HCl instillation and one received
1/3 normal saline instillation.

In the <u>positive control group</u> (n = 10), five
minutes before the HCl instillation, the rabbits received
either 0.9% NaCl (2 ml/kg body weight) or an irrelevant
monoclonal antibody (2 mg/kg body weight) intravenously.
The irrelevant monoclonal antibody was of the same
isotype as ARIL8.2 (IgG2a) and directed against the gp120
envelope protein on the human immunodeficiency virus.
Because there were no differences in the studied
parameters, the rabbits given the irrelevant monoclonal
antibody and those given NaCl intravenously were combined
into one group.

In the <u>pretreatment group</u> (n = 6), five minutes
before the HCl instillation, the rabbits received the
monoclonal antibody against IL-8 (ARIL8.2, 2 mg/kg body
weight) intravenously.

In the <u>treatment group</u> (n = 6), one hour after
the HCl instillation, the rabbits received ARIL8.2 (2
mg/kg body weight) intravenously.

In the <u>negative control group</u> (n = 4), five
minutes before the 1/3 normal saline instillation, the
rabbits received 0.9% NaCl (2 ml/kg body weight)
intravenously.

In the <u>positive control (long-term) group</u> (n =
3), one hour after the HCl instillation, the rabbits
received the irrelevant monoclonal antibody, anti-gp120
(2 mg/kg body weight) intravenously. The experiments
were planned for 24 h, however all rabbits in this group
died between 12-14 h after the HCl instillation.

In the <u>treatment (long-term) group</u> (n = 3), one
hour after the HCl instillation, the rabbits received

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ARIL8.2 (2 mg/kg body weight) intravenously. The rabbits were then studied for 24 h.

Hemodynamics, Airway Pressure, and Arterial Blood Gases

5   The heart rate, systemic blood pressure, and airway pressure were measured using calibrated pressure transducers (Pd23 ID, Gould Oxnard, CA., USA) and recorded continuously on a Grass polygraph (Grass Model 7 Polygraph, Grass Instruments, Quincy, MA., USA).

10 Arterial blood gases and pH and the systemic arterial pressure were measure every 30 minutes. The alveolar-arterial oxygen difference was calculated.

Extravascular Lung Water

15   Our method for the determination of extravascular lung water has been described previously in detail. Berthiaume et al. (1987) J. CLIN. INVEST. 79:335-343, and Wiener-Kronish et al. (1991) J. CLIN. INVEST. 88:864-875. In brief, the left lung was homogenized and

20 the extravascular lung water was determined by measuring the extravascular water-to-dry weight ratio (gram water/gram dry lung). Because the right lung was lavaged for cell counts, the data for extravascular lung water was obtained for the left lung only. The bronchoalveolar

25 lavage from the right lung and the homogenates from both lungs were used for measurement of radioactivity (see below).

Lung Vascular Permeability

30   For measurement of lung endothelial permeability to protein, the clearance of the vascular tracer protein, ^{131}I-albumin, across the endothelium into the extravascular compartments of the lungs was measured. The total extravascular ^{131}I-albumin accumulation in the

35 lung was calculated by taking the total lung ^{131}I-albumin (in lung homogenate and in the alveolar samples) and subtracting the vascular space ^{131}I-albumin. The [131]I-

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albumin in the vascular space was calculated by multiplying the counts in the final plasma sample by the calculated plasma volume in the lungs, as we have done previously. Berthiaume et al. (1987) J. CLIN. INVEST.

5   79:335-343, and Wiener-Kronish et al. (1991) J. CLIN. INVEST. 88:864-875. The extravascular accumulation of ^{131}I-albumin in the lung was expressed as plasma equivalents, or the milliliters of plasma that would account for the radioactivity in the lung.

10

Measurement of Free IL-8 Concentrations

            The concentrations of free IL-8, not bound by the anti-IL-8 monoclonal antibody, were measured by ELISA in plasma and in the final alveolar sample, as described.

15   Broaddus et al. (1994) J. IMMUNOL. 152:2960-2967. In this assay ARIL8.2 was used as the primary capture mAb so that it would not capture IL-8 already bound to ARIL8.2. Microtiter plates (96-well; ImmunoPlate MaxiSorp. Nunc. Alameda Chemical Sciences, Oakland, CA., USA) were coated

20   with ARIL8.2 (10 μg/ml), and then blotted dry and blocked with phosphate buffered saline (PBS) containing 0.5% bovine serum albumin (Sigma) for 1 h. Standards of rrIL-8 mixed with ARIL8.2, plasma samples and alveolar samples in several dilutions were added to the wells for a 1 h

25   incubation.   After washing, the secondary antibody (8Cl.1.6), conjugated to long-arm biotin (Biotin-S-NHS, Research Organics, Cleveland, OH., USA), was added for 2 h   followed   by   horseradish-peroxidase-conjugated streptavidin (1:5,000; Zymed Laboratories, South San

30   Francisco, CA., USA) for 1 h.   Tetramethyl benzidine (TMB;   2   component   system,   Kirkegaard   &   Perry Laboratories, Gaithersburg. MD., USA) was then added and color was allowed to develop in room temperature for 10 minutes. Optical density was then measured with an ELISA

35   plate reader at a wave length of 405 nm. Sample values were determined by interpolation using a 4-parameter program (Genentech Inc., South San Francisco, CA., USA)

from a standard curve generated over a range of 2,000 to 31 pg/ml. For the non-neutralized samples, the results for the dilutions were averaged over their linear range. However, when detecting antigen in the presence of a

5   soluble antibody identical to the capture antibody, an ELISA can be nonlinear at increasing dilutions, perhaps because antigen dissociates from the solutable antibody and is subsequently bound by the capture antibody. Therefore, for the neutralized samples, we chose the

10   lowest dilution (1:10) for quantitating free IL-8, knowing that this may still be an overestimate of the free IL-8 present. When testing the ELISA with standards of rrIL-8 mixed with ARIL8.2, we found that, as ARIL8.2 concentrations increased, the free IL-8 detected

15   decreased until, at a molar ratio of 5:1 and higher (mAb:IL-8), no IL-8 could be detected. All samples were coded so that the experimental condition was not known by the individual doing the assays.

20   **Statistical Analysis**

One-way ANOVA with repeated measurements analysis was used to compare samples obtained at several time points from the same animal. One-way ANOVA (factorial) was used when comparing other single groups.

25   Student-Newman-Keuls test was used as a post-hoc statistical test. Values are expressed as either mean ± SD or mean ± SEM as indicated in tables and figure legends. The data from the HCl instilled rabbits pretreated with either the irrelevant monoclonal

30   antibody, anti-gp 120, or with saline were combined because there were no significant differences between the groups.

RESULTS

35   **Oxygenation, Ventilation and pH**

In the short-term experiments, the alveolar-arterial oxygen tension differences in the

ARIL8.2 pretreatment and treatment groups were
significantly lower than in the positive control group by
2 h after the acid instillation and remained lower for
the 6 h experiment (Fig. 1A and Table 1). In both the
5 ARIL8.2 pretreatment and treatment groups, the
alveolar-arterial oxygen tension difference was not
significantly different from that in the negative control
group (Fig. 1A and Table 1).

Table 1. Oxygenation, ventilation, arterial pH, systemic blood pressure, heart rate, and airway pressure in the short-term experiments.

Condition	Alveolar-Arterial Oxygen Difference (mm Hg)	Arterial PCO₂ (mm Hg)	pH	Mean Systemic Arterial Pressure (mm Hg)	Heart Rate (beats/min)	Peak Airway Pressure (cm H₂O)
Positive Control Group (HCl), n = 10						
Baseline	107 ± 31	37 ± 4	7.44 ± 0.06	68 ± 13	300 ± 30	17 ± 3
6 h after instillation	390 ± 137*	67 ± 18*	7.19 ± 0.13*	56 ± 21	285 ± 23	26 ± 8*
Pretreatment Group (ARIL8.2, HCl), n = 6						
Baseline	107 ± 35	36 ± 4	7.44 ± 0.05	61 ± 13	307 ± 38	17 ± 2
6 h after instillation	136 ± 48†	38 ± 8*†	7.37 ± 0.04†	52 ± 10	276 ± 35	22 ± 4*
Treatment Group (HCl, ARIL8.2), n = 6						
Baseline	108 ± 40	32 ± 3	7.46 ± 0.08	63 ± 12	304 ± 34	16 ± 1
6 h after instillation	149 ± 40†	39 ± 5*†	7.36 ± 0.03†	52 ± 18	288 ± 28	23 ± 3*
Neutrophil Depletion Group (HCl), n+4						
Baseline	110 ± 54	30 ± 3	7.47 ± 0.07	64 ± 15	301 ± 23	16 ± 2
6 h after instillation	241 ± 64*†	33 ± 6†	7.36 ± 0.05†	53 ± 13	280 ± 19	22 ± 2*
Negative Control Group (1/3 Normal Saline), n = 4						
Baseline	109 ± 22	36 ± 2	7.41 ± 0.09	61 ± 4	300 ± 29	17 ± 2
6 h after instillation	108 ± 51†	41 ± 3*†	7.38 ± 0.05†	62 ± 12	287 ± 21	20 ± 2†

Data are means ± SD
• p<0.05 versus baseline
† p<0.05 versus positive control group

In the long-term experiments, the alveolar-arterial oxygen tension difference was significantly lower in the treatment (long-term) group than in the positive control (long-term) group by 2 h after HCl instillation (Fig. 1B and Table 2). In the positive control (long-term) group, the rabbits died with severe hypoxemia between 12-14 h. In the treatment (long-term) group, on the other hand, the rabbits lived for 24 h without hypoxemia.

Table 2. Oxygenation, ventilation, arterial pH, systemic blood pressure, heart rate, and airway pressure in the long-term experiments.

Condition	Alveolar-Arterial Oxygen Difference (mm Hg)	Arterial PCO$_2$ (mm Hg)	pH	Mean Systemic Arterial Pressure (mm Hg)	Heart Rate (beats/min)	Peak Airway Pressure (cm H$_2$O)
Positive Control (long-term) Group (HCl), n = 3						
Baseline	110 ± 12	31 ± 2	7.42 ± 0.05	55 ± 16	292 ± 7	18 ± 2
6 h after instillation	484 ± 88*	68 ± 1*	7.22 ± 0.03*	59 ± 23	284 ± 18	28 ± 4*
12 h after instillation'	504 ± 115*	87 ± 19*	7.12 ± 0.10*	48 ± 7	256 ± 30	31 ± 5*
Treatment (long-term) Group (HCl, ARIL8.2), n = 3						
Baseline	112 ± 18	30 ± 4	7.42 ± 0.02	56 ± 8	284 ± 7	17 ± 1
6 h after instillation	152 ± 19*†	33 ± 2*†	7.36 ± 0.06†	57 ± 4	288 ± 21	21 ± 1*†
12 h after instillation	122 ± 14†	33 ± 2*†	7.34 ± 0.04*†	54 ± 4	280 ± 18†	21 ± 1*†
24 h after instillation	161 ± 29*	30 ± 2	7.36 ± 0.01*	49 ± 6	272 ± 14	21 ± 3*

Data are means ± SD

*   p<0.05 versus baseline

†   p<0.05 versus positive control (long-term) group

'   all rabbits in this group died between 12-14 h

The acid-induced abnormalities in $PaCO_2$ and pH were prevented by either pretreatment or treatment with ARIL8.2 both in the 6 h and 24 h studies as well as by neutrophil depletion (Table 1 and Table 2).

Extravascular Lung Water

In the short-term experiments, the extravascular lung water (water-to-dry weight ratio) in the pretreatment and treatment groups was 35% lower than in the positive control group and not significantly different from that in the negative control group at 6 h (Fig. 2A). In the long-term experiments, the extravascular lung water in the treatment (long-term) group at 24 h was 100% lower than in the positive control (long-term) group at 12-14 h (Fig. 2B).

Lung Vascular Permeability

In the short-term experiments, the extravascular accumulation of plasma equivalents in the lungs of the pretreatment and treatment groups was 70% lower than in the positive control group at 6 h (Fig. 3A), although the extravascular accumulation of plasma was significantly higher than in the negative control group. In the long-term experiments, the extravascular accumulation of plasma equivalents in the lung was 75% lower in the treatment (long-term) group at 24 h than in the positive control (long-term) group at 12-14 h (Fig. 3B).

Systemic Blood Pressure, Heart Rate, and Peak Airway Pressure

No differences were observed in the blood pressure or heart rate at any time among the experimental groups (Table 1). The peak airway pressure rose in all groups within 5 minutes after instillation. While the airway pressure in the positive control group remained high, the airway pressure in the negative control group

decreased by 6 h. In both the pretreatment and treatment
groups, the airway pressure tended to decrease, although
this did not reach statistical significance (Table 1).
Similar findings were observed in the long-term studies
5   (Table 2).

**Cell Counts in Bronchoalveolar Lavage Fluid and in
Peripheral Blood**

              In the short-term experiments, the number of
10   polymorphonuclear leukocytes (PMN) lavaged from the air
spaces in the pretreatment and treatment groups was more
than 50% lower than in the positive control group and no
different from that in the negative control group (Fig.
4A). In the long-term experiments, the number of lavaged
15   neutrophils in the treatment (long-term) group at 24 h
was more than 75% lower than in the positive control
(long-term) group at 12-14 h (Fig. 4B). No significant
differences   were   seen   in   the   number   of   alveolar
macrophages   between   the   different   groups   (data   not
20   shown). In the peripheral blood, there was an identical
small increase in the neutrophil count in all groups,
except   in   the   neutrophil-depleted   group,   where   no
circulating neutrophils were observed at any time during
the experiment.
25

**Concentrations of Free Interleukin-8 Plasma, Alveolar
Fluid, and Bronchoalveolar Lavage Fluid**

              In   the   short-term   experiments,   the
concentrations of free IL-8 in the final alveolar fluid
30   samples were more than 10-fold lower in the pretreatment
and treatment groups than in the positive control group
at 6 h (Table 3). Because no undiluted alveolar fluid
could be aspirated in the negative control group, free
IL-8 was also measured in bronchoalveolar lavage fluid.
35   As   was   the   case   in   the   alveolar   fluid,   free   IL-8
concentrations in the lavage fluid were more than 10-fold
lower   in   the   pretreatment   and   treatment   groups   than   in

the positive control group (Table 3). Of interest, the free IL-8 in lavage fluid in the treatment group was not different from the negative control group (Table 3). In the long-term experiments, alveolar fluid could not be

5 aspirated from the treatment (long-term) group. However, in the lavage fluid, the free IL-8 concentrations were significantly lower in the treatment (long-term) group than in the positive control (long-term) group (Table 3). In the plasma, the concentration of immunoreactive free

10 IL-8 (not bound by the anti-IL-8 monoclonal antibody, ARIL8.2) was low at all times and similar for all groups (330 ± 28 pg/ml).

Table 3. Free rabbit IL-8 concentrations (IL-8 not bound by the anti-IL-8 monoclonal antibody, ARIL8.2) in alveolar fluid and lavage fluid in the various experimental groups.

Condition	Time of sampling	IL-8 concentrations in Alveolar Fluid (ng/ml)	IL-8 concentrations in Lavage Fluid (ng/ml)
Positive control group (HCl)	6 h	40.5 ± 18.2 (8)	6.7 ± 2.5 (4)
Pretreatment group, (HCl) (ARIL8.2)	6 h	3.3 ± 1.9* (6)	nc
Treatment group (HCl) (ARIL8.2)	6 h	3.0 ± 1.7* (6)	0.6 ± 0.3* (3)
Negative control group (1/3 normal saline)	6 h	na	1.4 ± 1.6* (3)
Positive control (long-term group (HCl)	12-14 h	29 ± 2.2 (3)	2.1 ± 2.8 (3)
Treatment (long-term) group (HCl, ARIL8.2)	24 h	na	0.2 ± 0.2† (3)

* $p < 0.05$ versus positive control group;
† $p < 0.05$ versus positive control (long-term) group
na   sample not possible to obtain
nd   not determined
Number of determinations within parenthesis
Values are means ± SD

DISCUSSION

The results of this study confirm that acid aspiration-induced lung injury is primarily mediated by
5   neutrophils recruited to the lung by IL-8. First, in the rabbits instilled with acid, large numbers of neutrophils were recovered from the air spaces in association with markedly elevated quantities of IL-8. The nearly undetectable concentrations of IL-8 in the plasma in all
10 groups supports the premise that IL-8 is generated

locally in the lung following acid aspiration. The
concentrations of IL-8 in the air spaces 6 h after acid
aspiration (41 ± 18 ng/ml.) are biologically relevant; in
in vitro assays of chemotaxis and neutrophil priming, IL-
5   8 is biologically active at ten-fold lower concentration.
Second, the role of the neutrophil in injuring the lung
was supported by the marked reduction of lung injury
after neutrophil depletion. Third, the role of IL-8 in
mediating the recruitment of neutrophils and the
10   resultant lung injury following acid instillation was
established by neutralizing IL-8. The anti-IL-8 antibody
effectively reduced the concentrations of free IL-8 to
less than 10% of the concentration in the positive
control (acid-instilled) rabbits, to a level at the lower
15   limit of the biological activity of IL-8 in both *in vitro*
and *in vivo* studies. This IL-8 concentration was
probably close to that in the negative control (saline-
instilled) rabbits, as judged by the similar IL-8
concentrations in the bronchoalveolar lavage fluid. The
20   use of the anti-IL-8 monoclonal antibody led to a more
than 50% decrease in the neutrophil influx and, more
importantly, to a dramatic reduction in the severity of
the acute lung injury due to acid aspiration. Following
neutralization of IL-8, the acid-induced abnormalities in
25   gas exchange, extravascular lung water, and lung vascular
permeability were nearly completely prevented.

The three separate indices of lung injury
demonstrated internally consistent and convincing results
in both the short-term (6 h) and the long-term (24 h)
30   studies. First, the alveolar-arterial oxygen tension
difference was nearly normal in acid-instilled rabbits
given the anti-IL-8 monoclonal antibody, indicating the
absence of alveolar edema. Second, when IL-8 was
neutralized, the extravascular lung water in acid-
35   instilled rabbits was not different from that in rabbits
instilled with saline alone. The water-to-dry weight
ratio of 4.2-4.4 g water/g dry lung in the pretreatment

and treatment groups and in the saline-instilled control
group is most consistent with mild interstitial edema.
In the positive control group, on the other hand, the
water-to-dry weight ratio of 7.0 g water/g dry lung at
5   6 h and 8.0 g water/g dry lung at 12-14 h clearly
indicates significant alveolar edema. These differences
become more obvious when the lung water is expressed as
the calculated milliliters of water accumulated in the
lung in excess of that in a normal rabbit lung (3.2 g
10 water/g dry lung). In the positive control group at 6 h,
the excess water in both lungs was approximately 9.2 ml,
or more than three-fold higher than the amount
(1.6-2.6 ml) in the negative control, pretreatment and
treatment groups. Finally, the endothelial barrier was
15 significantly protected in the acid-instilled rabbits
given the anti-IL-8 monoclonal antibody. There was a
small increase in lung endothelial permeability that was
not prevented by pretreatment or treatment with the
monoclonal antibody to IL-8. This increase, however, was
20 not sufficient to cause a net accumulation in
extravascular lung water. A small increase in lung
endothelial permeability without an accompanying increase
in lung water has previously been described in sheep
given an endotoxin infusion. Overall, once IL-8 was
25 neutralized, the effects of acid on the lung were not
different from the effects of saline alone.

            The fact that the anti-IL-8 monoclonal antibody
was equally effective when given 1 h after the acid
instillation as when administered 5 minutes before the
30 injury confirms that the development of lung injury is
delayed following acid instillation. Such a time course
is consistent with the kinetics expected for IL-8
expression. Studies in which IL-8 concentrations have
been measured sequentially *in vivo* demonstrate that
35 biologically relevant IL-8 concentrations are first found
2 h after an endotoxin stimulus. With additional time,
IL-8 concentrations may be amplified further by the

action of proximal macrophage-derived cytokines such as TNF-α or IL-1 on the nearby bystander cells, such as epithelial cells. The important role of proximal cytokines in acid-induced lung injury has been suggested
5   by an earlier study in which neutralization of TNF-α significantly reduced the injury caused by acid-aspiration. Since TNF-α is a major proximal cytokine leading to the production of IL-8 by many cells, neutralization of TNF-α appears earlier than IL-8 in the
10 inflammatory cascade, it is unlikely that anti-TNF-α would be effective as late as anti-IL-8 following acid-instillation. Another factor that may contribute to the relatively wide therapeutic window of anti-IL-8 (at least 1 h) is that after its production, IL-8 must also diffuse
15 to the endothelium and interact with neutrophils. Given the time necessary for IL-8 production and diffusion, it is conceivable that anti-IL-8 therapy given later than 1 h after acid aspiration would also be effective.

Although the data in this study indicates that
20 IL-8 is critical for the development of the acute injury following acid aspiration, it is likely that IL-8 mediates injury in conjunction with other proinflammatory molecules. When used alone, IL-8 appears to be a relatively weak neutrophil activator, although in vitro
25 studies indicate that it is an effective primer of activation by other mechanisms. In in vivo studies in which IL-8 alone was injected into normal human skin, neutrophils were recruited without the appearance of wheal or flare. Similarly, when IL-8 alone was instilled
30 into a normal tracheal segment in dogs, neutrophils were recruited without releasing elastase or lysozyme. In contrast to these studies in which IL-8 was used alone, in acid aspiration lung injury, IL-8 is undoubtedly generated in conjunction with other cytokines, such as
35 TNF-α and IL-1, and other inflammatory mediators, such as leukotrienes, complement fragments, and platelet activating factor. However, in spite of the different

cytokines and inflammatory mediators generated following acid aspiration into the lung, inhibition of IL-8 alone is capable of preventing the experimental lung injury generated by acid aspiration in rabbits.

5   Aspiration of gastric contents is a major clinical cause of morbidity and mortality and effective therapy for this condition is currently unavailable. Current management is limited to positive pressure ventilation and careful management of fluid therapy. The 10 data in this study suggest a promising therapeutic potential for anti-IL-8 therapy for this condition. There are several possible advantages of this approach. Because IL-8 is a distal cytokine, its neutralization may have more limited effects than neutralization of a more 15 proximal, pluripotent cytokine, such as TNF-α. Also, neutralization therapy might be required only for a short time, during the time that IL-8 is generated. In *in vivo* studies following endotoxin stimulation, IL-8 concentrations have returned towards normal in less than 20 12 hours. In the long-term studies at 12-14 h following acid aspiration, alveolar fluid IL-8 concentrations in untreated rabbits were significantly lower than at 6 h, suggesting that a need for long-term anti-IL-8 therapy following a single acid aspiration might be unnecessary. 25 The time of onset of the aspiration would be known with certainty in many cases because gastric aspiration is frequently witnessed, whereas in other clinical conditions, such as sepsis, the time of onset is often difficult to identify. And, most importantly, the delay 30 in onset of the acid-aspiration injury allows a clinically feasible therapeutic window of at least one hour. A potential limitation of anti-IL-8 therapy is that, like any anti-inflammatory therapy, it might inhibit the host immunity and increase the risk of 35 infection. Indeed, secondary bacterial pneumonia is a known complication of acid aspiration, occurring 2-10 days after aspiration.

27

While the above is a complete description of the preferred embodiments of the invention, various alternatives, modifications, and equivalents may be used. Therefore, the above description should not be taken as

5   limiting the scope of the invention which is defined by the appended claims.

28

WHAT IS CLAIMED IS:

1.   A method for treating a host having lungs exposed to an acid medium, said method comprising administering to said host an amount of an anti-IL-8 binding substance effective to inhibit damage to the lungs resulting from such exposure.

2.   A method as in claim 1, wherein the anti-IL-8 binding substance binds to the IL-8-receptor-binding region on IL-8.

3.   A method as in claim 1, wherein the anti-IL-8 binding substance is anti-IL-8 antibody which binds to the IL-8-receptor-binding regions on IL-8.

4.   A method as in claim 1, wherein the anti-IL-8 binding substance is administered intravascularly or by pulmonary delivery.

5.   A method as in claim 1, wherein the anti-IL-8 binding substance is administered within one hour of exposure to acid medium.

6.   A method as in claim 1, wherein the amount of anti-IL-8 binding substance is sufficient to neutralize IL-8 binding to neutrophils.
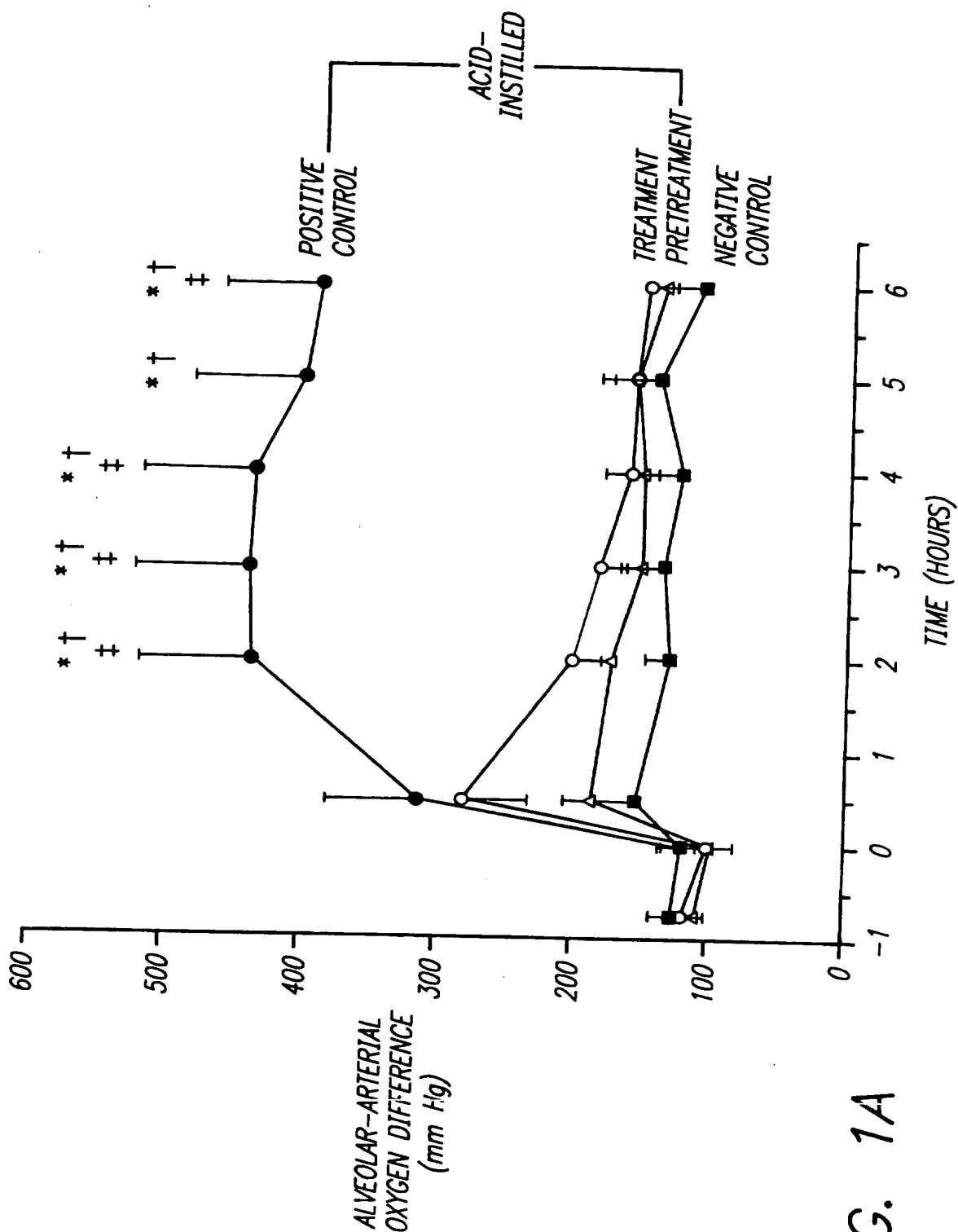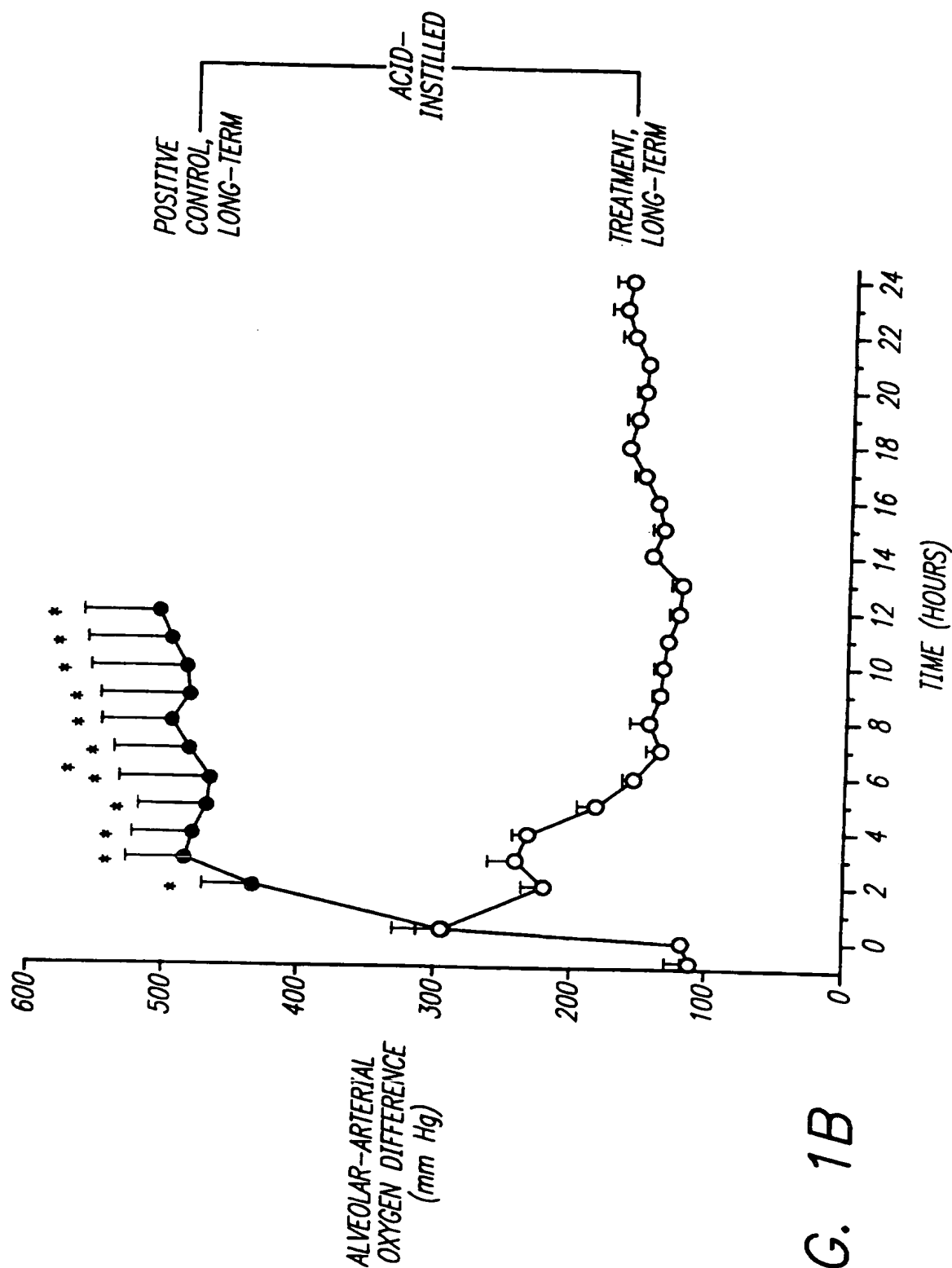
FIG. 1A

FIG. 1B

FIG. 2A
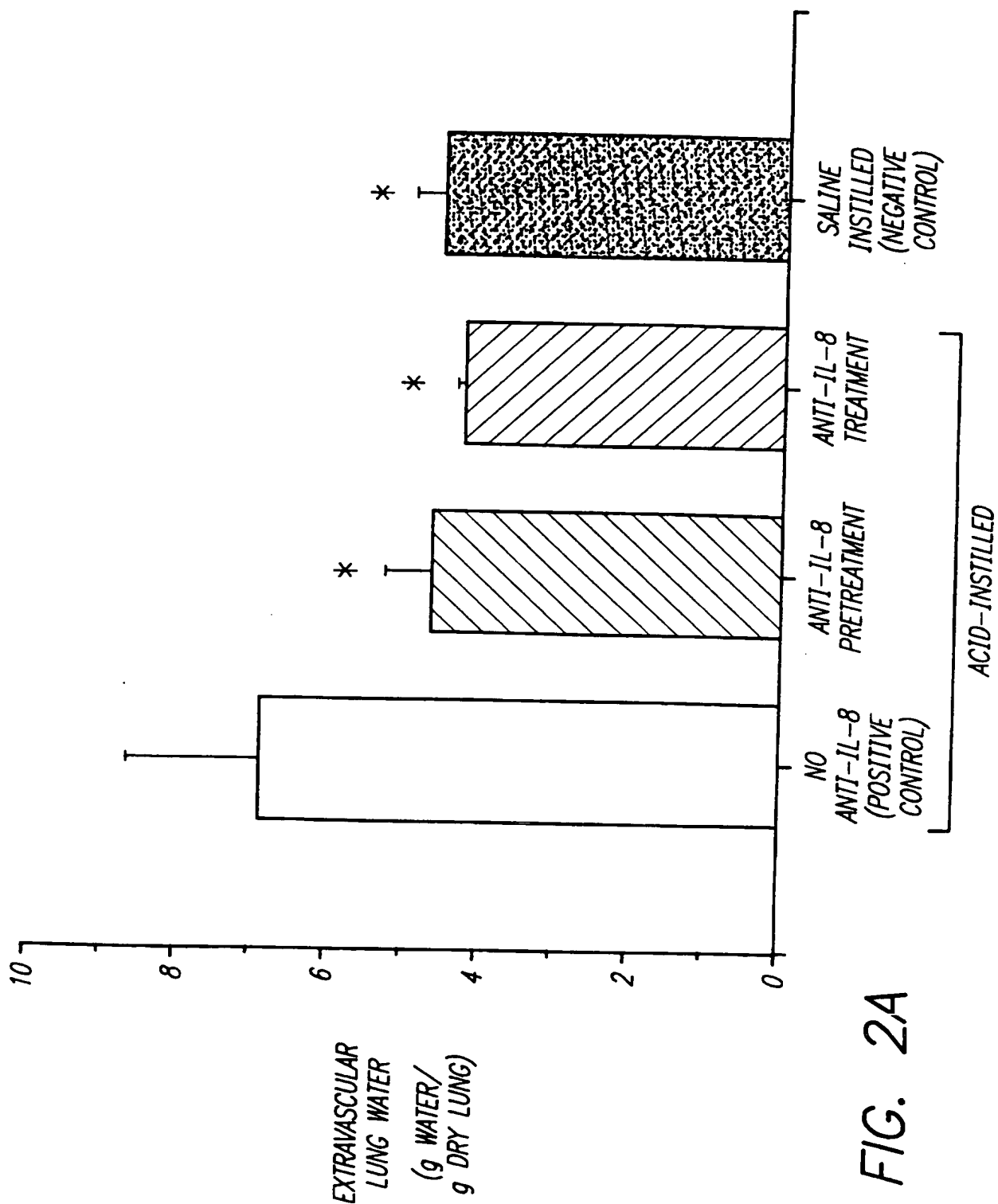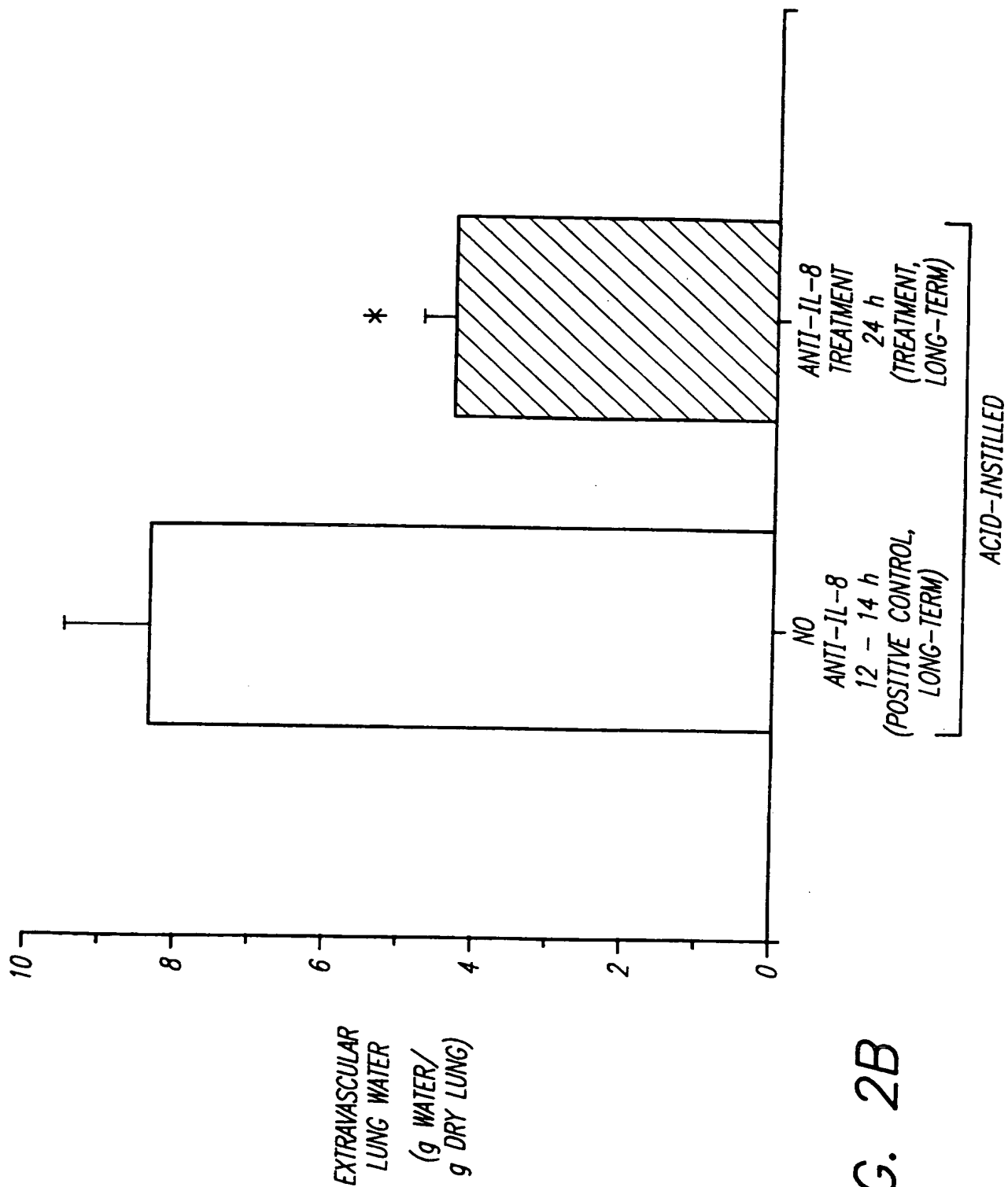
FIG. 2B
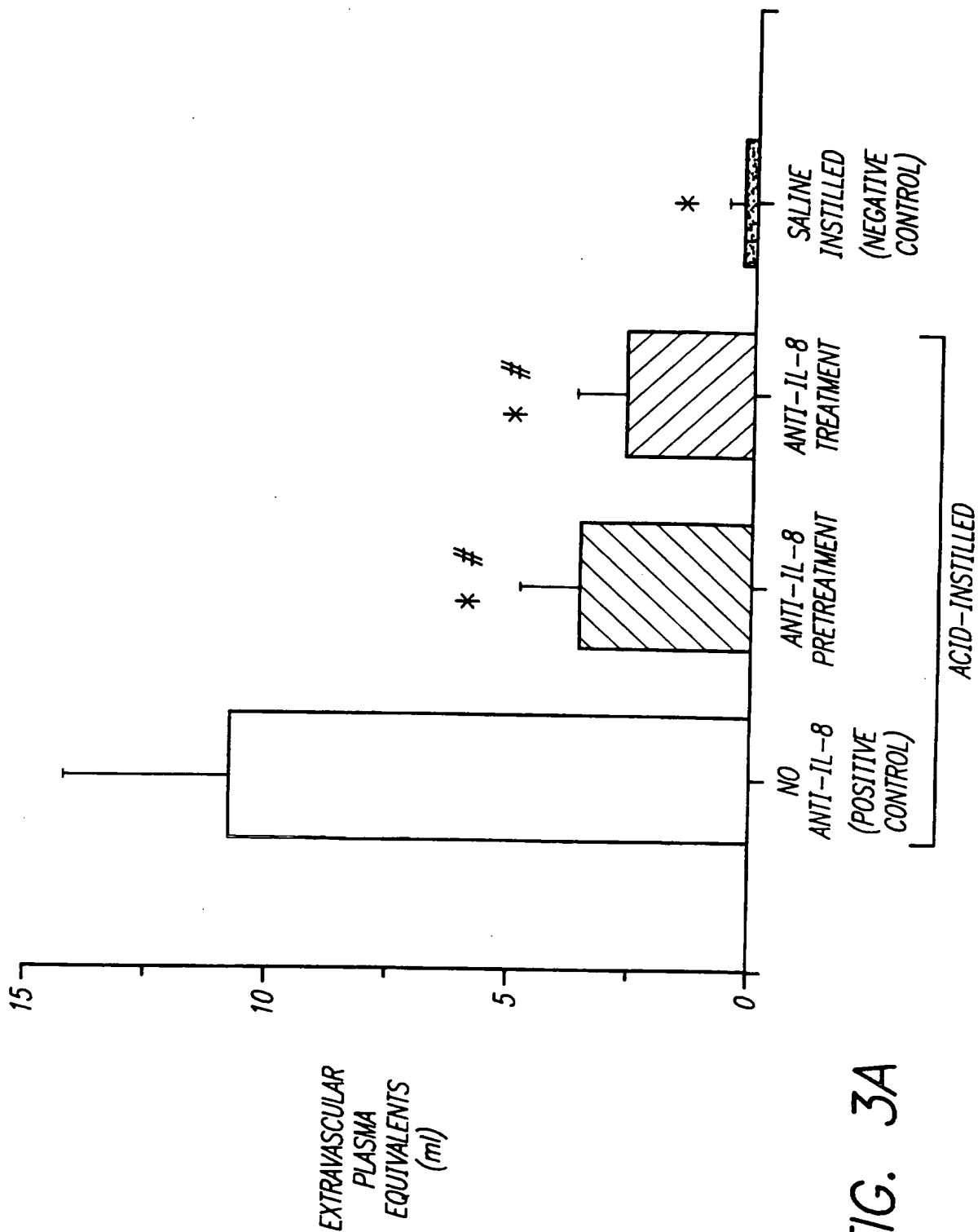
FIG. 3A
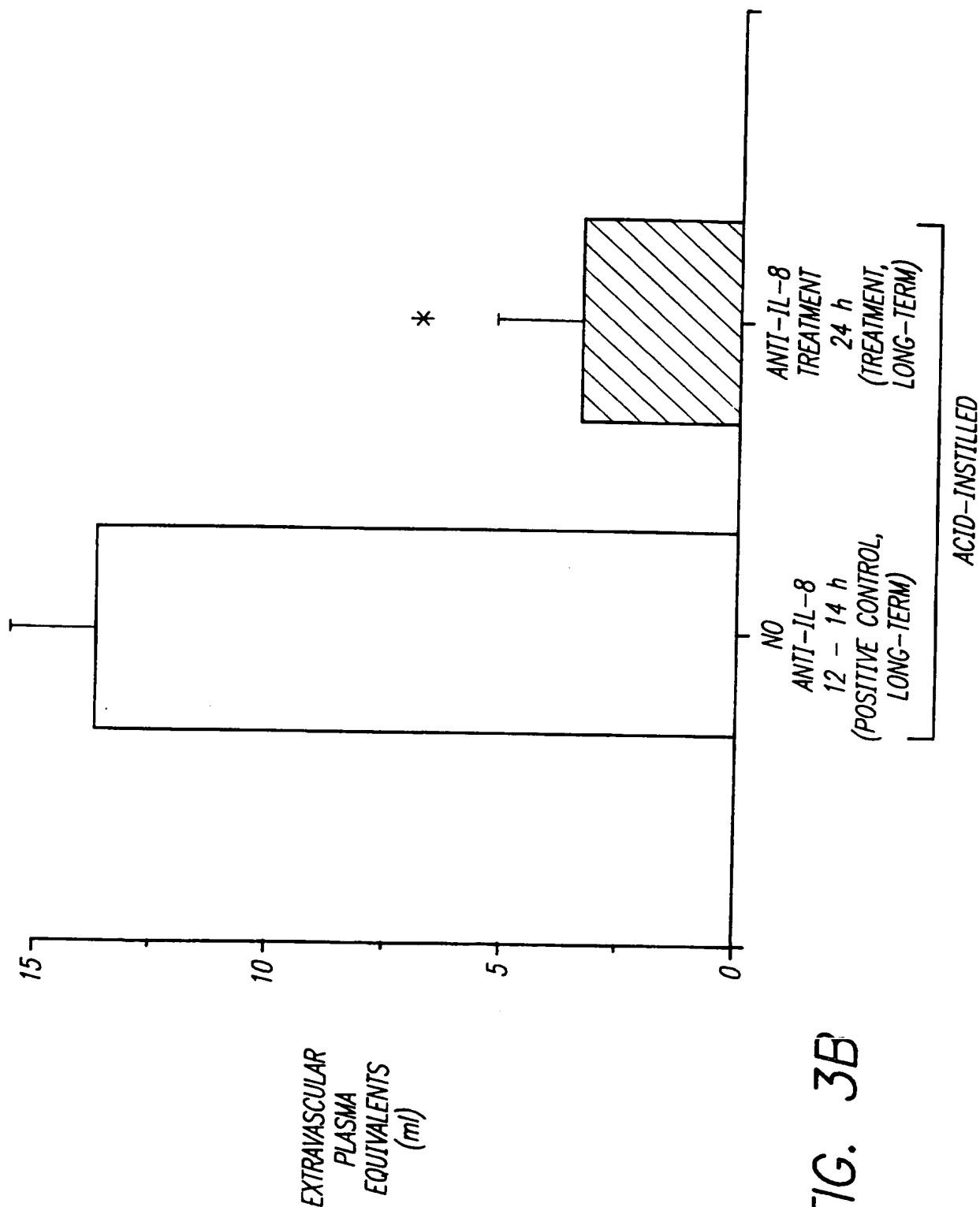
FIG. 3B
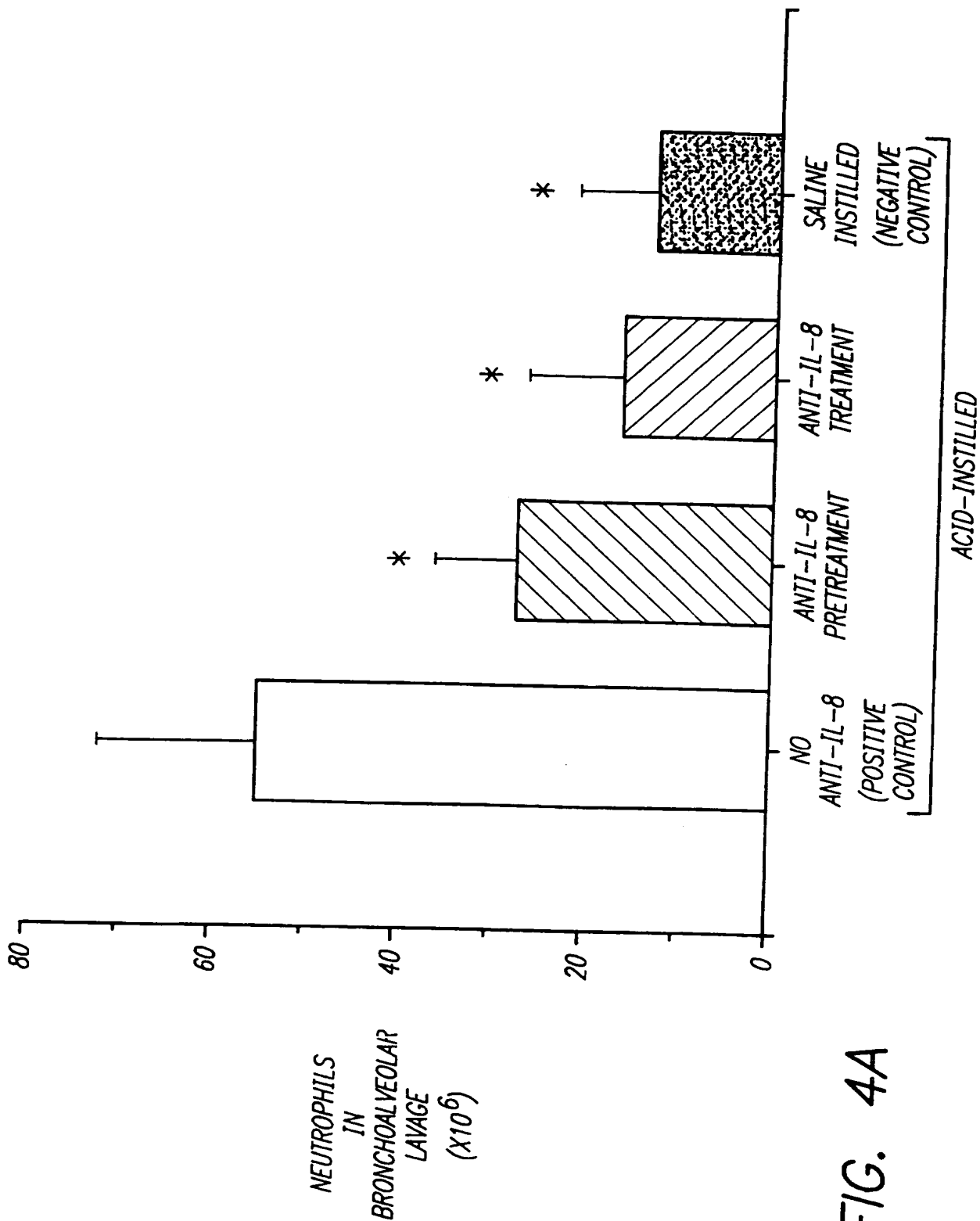
FIG. 4A

FIG. 4B

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :Please See Extra Sheet.

US CL   :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   424/131.1, 145.1, 158.1, 172.1; 514/2; 530/350, 388.23, 389.2, 351

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, EMBASE, DERWENT WPI, CHEM AB, APS search terms: author names, IL-8, lungs, acid, antibody, anti IL-8, receptor, acid, neutrophils, binding

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SEKIDO et al. Prevention of lung reperfusion injury in rabbits by a monoclonal antibody against interleukin-8. Nature. 14 October 1993, Vol. 365, pages 654-657, see entire document.	1-6
Y	GOLDMAN et al. Tumor necrosis Factor-alpha mediates acid aspiration-induced systemic organ injury. Ann. Surg. October 1990, vol. 212, pages 513-520, see entire document.	1-6

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
		"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search

08 MAY 1996

Date of mailing of the international search report

13 MAY 1996

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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 38/00, 38/16, 39/395; C07K 14/00, 14/715, 14/435, 16/00, 16/24, 16/28

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/131.1, 145.1, 158.1, 172.1; 514/2; 530/350, 388.23, 389.2, 351